Model for capping of membrane receptors based on boundary surface effects

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ABSTRACT Crosslinking of membrane surface receptors may lead to their segregation into patches and then into a single large aggregate at one pole of the cell. This process is called capping. Here, a novel explanation of such a process is presented in which the membrane is viewed as a supersaturated solution of receptors in the lipid bilayer and the adjacent two aqueous layers. Without a crosslinking agent, a patch of receptors that is less than a certain size cannot stay in equilibrium with the solution and thus should dissolve. Patches greater than a certain size are stable and can, in principle, grow by the precipitation of the remaining dissolved receptors from the supersaturated solution. The task of the crosslinking molecules is to form such stable patches. These considerations are based on a qualitative thermodynamic calculation that takes into account the existence of a boundary tension in a patch (in analogy to the surface tension of a droplet). Thermodynamically, these systems should cap spontaneously after the patches have reached a certain size. But, in practice, such a process can be very slow. A suspension of patches may stay practically stable. The ways in which a cell may abolish this metastable equilibrium and thus achieve capping are considered and possible effects of capping inhibitors are discussed.

In recent years it has been demonstrated that plasma membranes, being composed of lipid bilayers, have fluid characteristics and may allow the lateral motion of proteins embedded in them (1, 2). These proteins can move in the plane of the membrane unless this motion is restricted—for example, by interaction with cytoplasmic structures. Thus, proteins embedded in the membrane can be distributed uniformly in the plane of the membrane unless there are restrictions.

Investigations by Taylor et al. (3) have shown that molecules that can crosslink proteins (receptors) on the cell surface [e.g., anti-immunoglobulin (anti-Ig), concanavalin A (Con A)] may cause redistribution of these proteins into segregated regions, many patches, or one cap. Much work has been done on such processes in various cell types described extensively in the recent review by Schreiner and Unanue (4). A capping process is usually viewed as composed of two distinct stages: first, patches of crosslinked receptors are seen; then, patches migrate into one region on the cell to form a cap.

There have been few explanations of why and how caps are formed (5-11). As has been suggested (4, 12), not all capping processes need to be alike; they might consist of quite different mechanisms. Thus, for different cells, receptors, and conditions, one might anticipate different mechanisms leading to segregation of receptors from the membrane into a cap or patch. An aspect that has not yet been considered is the role of boundary effects on capping processes. Proteins on a boundary between a membrane patch and the membrane at large experience different intermolecular forces from those experienced by a protein molecule well inside the patch. This difference creates an extra tension on the boundary layer of the patch and thus raises the free energy of the patch. As is explained later, the existence of the boundary tension causes patches that are less than a certain size to be thermodynamically unstable. Thus, a membrane may contain single dissolved proteins beyond the saturation level without the precipitation of the dissolved proteins from the two-dimensional solution (the membrane which contains the lipid bilayer and the adjacent two thin aqueous layers where parts of the protein molecules may be also embedded) into a separate phase (patches or a cap). This effect is analogous to the surface tension of a bubble in three dimensions.

The role of the crosslinking agent is to form artificially stable patches of the right size on which the proteins in membrane solution can precipitate.

This paper considers the influence of these boundary effects on capping processes.

STABILITY CRITERION FOR A PATCH

If there are a few receptors (protein molecules) on the cell surface, they might be mixed with the lipid molecules in one phase, forming a two-dimensional solution. By a protein molecule is meant a bare protein molecule, or when necessary, a protein molecule with solvated lipid and water molecules. At a given temperature the solubility is a certain concentration (number of receptors per unit area) of receptors dissolved in the two-dimensional solution that can stay in equilibrium with a precipitate or a patch (a condensed phase) with infinite dimensions. If the concentration of the receptors in the solution is higher than this critical concentration, they should, in principle, leave the solution and thus precipitate in the condensed phase. If the receptor concentration is made lower than this critical concentration, receptors will leave the condensed phase and enter the solution, thus increasing the receptor concentration in the solution. This situation is analogous to a solid (the condensed phase) in three dimensions that can stay in equilibrium with its solution (the dilute phase) at a certain concentration (whose magnitude depends on temperature).

The precipitation, or the accumulation into a separate phase, has to occur by, first, the formation of small aggregates of receptors (patches) or, in other words, small regions of receptor-dense phase. Each of the receptor molecules that is well inside

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Abbreviations: Con A, concanavalin A; anti-Ig, anti-immunoglobulin.
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the patch is surrounded by molecules of the same type. On the other hand, receptor molecules at the boundary between the patch and the lipid dilute phase are exposed also to molecules of the dilute phase. Therefore, the receptor molecules on the boundary exercise different intermolecular forces from those receptor molecules well inside the patch, thus creating a boundary tension. This boundary tension is analogous to the well-known surface tension of droplets in systems of three dimensions. The existence of a boundary tension in a patch complicates the conditions under which a patch can stay in equilibrium with a solution of its receptors.

The change in the Gibbs free energy when receptor molecules dissolved in the membrane (the dilute phase) transfer into a patch (the condensed phase) is composed of the following two parts [in analogy to the three-dimensional case(13)].

1. The first contribution to the change is due to the difference in the Gibbs free energy between receptors dissolved in the dilute phase and receptors in the condensed form. (This part is analogous to the difference in free energy between molecules in the vapor and the liquid state.) Using simple and qualitative thermodynamic considerations, one finds that this contribution to the change in the free energy when  \( n \) mol of receptors are transferred from the bulk of the membrane into a condensed phase is:

\[
\Delta G_1 = -\pi \gamma \rho (\rho/M)RT \ln X
\]

in which \( n = \pi \rho^2 / M \), \( r \) is the radius of the patch formed from the \( n \) mol of receptors, \( \rho \) is the receptor density in the patch (mass per unit area), \( R \) is the gas constant, \( T \) is the absolute temperature, and \( X \) is approximately \( c /c_m \), \( c_m \) being the solubility of the receptors in the membrane and \( c \) their actual concentration. If \( c > c_m \), the solution is supersaturated. Under this condition, \( c /c_m > 1 \) and \( \ln X \) is positive. Because of the minus sign, this term is negative. This is intuitively understood because, neglecting the effects of the boundary tension, when the solution is supersaturated the receptors will tend to precipitate into a condensed phase in order to reach lower values of the free energy.

2. The second contribution to the change in free energy when \( n \) mol of receptors are transferred from the solution into a condensed phase is due to the existence of the boundary tension on the border between the patch and the solution. The patch has an additional energy, the boundary energy, with the magnitude \( \Delta G_2 = 2\pi \gamma r \). \( 2\pi r \) is the circumference of the patch and \( \gamma \) is the interfacial free energy per cm of the boundary between the patch and the membrane. This contribution is positive.

The sum of the two parts,

\[
\Delta G = \Delta G_1 + \Delta G_2 = -\pi \gamma \rho (\rho/M)RT \ln X + 2\pi \gamma
\]

is the change in the Gibbs free energy when \( n \) mol of receptors are transferred from the solution to form a patch of a condensed phase. It is to be noted again that the second term is positive whereas if the solution is supersaturated, the first term is negative. Thus, if at small \( r \) the second term is dominant, \( \Delta G \) is positive. At large \( r \), on the other hand, the first term becomes dominant and thus \( \Delta G \) is negative. A typical plot of \( \Delta G \) as a function of the patch radius is given in Fig. 1. This plot has the appearance of an activation energy plot. \( \Delta G \) as a function of the patch radius starts with a value of 0 (for \( r = 0 \)). Then it increases with \( r \) up to a maximum when the patch radius has the critical value of \( r_c \),

\[
r_c = \gamma M / (\rho RT \ln X).
\]

\( \Delta G \) decreases as \( r \) increases for \( r > r_c \) and reaches negative values. Thus, a patch with \( r < r_c \) is unstable because, in order to decrease \( G \), it will dissolve. Once there is a patch with \( r > r_c \), it will grow in order to decrease \( G \). A patch with \( r = r_c \) for which \( \Delta G \) is maximal, can stay in equilibrium with receptors in the solution. But this is an unstable equilibrium because a small fluctuation can cause a shift either to the right (increasing the patch) or to the left (dissolving the patch).

To sum up, the dependence of \( \Delta G \) on \( r \) gives the following stability criterion for a patch: Given a membrane supersaturated with receptors (\( c > c_m \)), a patch with \( r < r_c \) is unstable and thus dissolves and disappears, but a patch with \( r > r_c \) will increase in size.

If the conditions are such that \( r_c \) corresponds to a patch containing more than a few receptors, it is unlikely that a natural fluctuation will form a patch with a \( r > r_c \). So the supersaturated solution of the receptors in the membrane might stay in this state. If, by some artificial means, a patch with \( r > r_c \) is formed in the membrane, it will grow according to the above stability condition.

A way to form a patch is by crosslinking receptors by multivalent molecules such as Con A, anti-Ig, etc. Once the radius of the patch formed by cross-linking agents achieves the magnitude of \( r_c \), additional receptors, dissolved in the bulk of the membrane, can condense on it spontaneously. Of course, the crosslinking can still go on, but free receptors (or labeled ones) are now able to condense on the nucleus of the crosslinked patch and thus decrease the Gibbs free energy.

The argument presented in this section is qualitative. The shape of the patches is taken as round discs. In practice, their shape might be irregular, which would complicate the calculation of \( \Delta G \). If the conditions are such that the crosslinking event produces a continuous network of crosslinked receptors...
over the cell, then discrete patches are not produced and capping should not occur spontaneously. This is an experimental condition employed by Woll et al. (14) in a study of crosslinking of dextran derivatives in planar bilayer membranes.

DISCUSSION

The qualitative thermodynamic consideration illustrates why capping does not occur spontaneously—patches under a critical size are unstable. The effect of crosslinking of the receptors (by crosslinking molecules) is to form nuclei (patches) of such sizes that the remaining free receptors can condense on them spontaneously, leading to a large condensed region—a cap. After the cap is formed it can be further stabilized by an active interaction with cytoskeleton elements and also endocytized.

The capping process described here may be one of the many capping processes that occur in various cells and receptors (4, 12). This mechanism might take place only in systems that are supersaturated with receptors. The number of receptors measured per cell ranges from \( \sim 10^4 \) to \( \sim 10^6 \) (cf. Stackpole et al. (15)) in cells that can undergo capping. Taking the number of receptors per cell as \( 10^4 \) (lower limit), the receptor's molecular weight as \( 10^5 \), the cell diameter as \( 10 \mu m \), and the distance in which the center of mass of the receptor can move vertically as \( 10 \AA \) (16), one obtains a lower limit for the receptor concentration of about \( 5.3 \times 10^{-9} g/ml \). This is quite a high concentration compared with typical solubilities of proteins in water. Thus, it is likely that in some cells the membrane with the two adjacent aqueous layers is a supersaturated solution of the receptors to be capped. Such a supersaturated solution might stay in this state because the formation of a large cluster requires the diffusion of receptors to the location where the cluster is forming. Because part of the receptor is embedded in the two-dimensional lipid matrix, its diffusion along the membrane is slower by a few orders of magnitude than in water. Thus, until a sufficient number of receptors diffuse to a forming cluster spot, an unstable cluster may disintegrate. Therefore, the chance of forming a cluster greater than the critical size can be low, and the solution can stay supersaturated. Recent work by Cohen and Eisen (16) discusses another important effect of the high effective concentrations of surface molecules in membranes.

An unknown parameter is the boundary tension, \( \gamma \). Taupin et al. (17) used a similar approach to study osmotic pressure-induced pores in phospholipid vesicles. For that system the boundary tension is at the boundary of a pore in the vesicle through which water can flow. Thus, the interface includes a contact between the hydrophobic parts of the phospholipid molecules and the hydrophilic aqueous phase. The values they obtained for \( \gamma \) are \( 5.5 \times 10^{-7} < \gamma < 8.0 \times 10^{-7} \) dyne. For the present situation the interface between a patch and the membrane contains molecules of a more similar nature than in the case of the pores. Both the proteins and the phospholipids are amphipatic and, in the interface, their hydrophobic parts are against each other whereas the hydrophilic parts of the proteins are adjacent to the aqueous phases and to the phospholipid hydrophobic parts. Thus, \( \gamma \) should be lower than in the phospholipids/water interface. If one takes \( \gamma \) as \( 10^{-8} \) dyne, in \( x \) as of the order of 1, \( T = 300 \) K, the number of receptors per cell as \( 10^4 \) (lower limit), and \( 10 \mu m \) for the diameter of the cell, a reasonable approximate value of \( 7.6 \times 10^{-22} c m \) is obtained for \( r_c \) (through Eq. 3), the critical radius of a patch.

A few more points should be made. First, it was observed by Taylor et al. (5), Unanue et al. (18), Karmovsky et al. (19), and Stackpole et al. (15) that low concentrations of the crosslinking agent (e.g., anti-lg) in the medium surrounding the cells might not produce efficient capping. This can be understood in terms of the above considerations because low concentrations of the crosslinking molecules in the medium might not be enough to produce crosslinked patches of an adequate size and thus capping will not occur.

Second, Loor et al. (20) have suggested that, after patches are formed, they are stable even if the external crosslinks are chemically cleaved. This is understood by the above-mentioned stability criterion—once a patch with a magnitude greater than the critical size is formed by crosslinking, it is stable even if the crosslinks are chemically cleaved.

Third, on a patch greater than the critical size the receptors can accumulate spontaneously. These receptors can be either free (unattached), crosslinked, or labeled. By labeled receptors one means receptors that are connected to a crosslinking molecule not attached to any other receptor molecule.

Recent work (21, 22) has shown that, in some cells and receptors, actin and myosin aggregate under patches and caps. The considerations expressed in this work can be applied also to systems in which actin and myosin are directly or indirectly connected to single or to crosslinked receptors, when the actomyosin components do not take an active role in the collection of the receptors and patches into the cap. A direct or indirect connection of receptors and patches to actin and myosin may change the value of the boundary tension, \( \gamma \), and thus affect the capping process described here. For such a case the two-dimensional solution is composed of the lipid bilayer and the two adjacent aqueous layers, one of which contains also a part or of the whole actomyosin system connected to the receptors and patches.

It should be emphasized that the above stability criterion is thermodynamic rather than kinetic. This means that, in principle, systems in which this criterion prevails should cap spontaneously after patches greater than the critical size have been formed. But, in practice, the rate at which such cells cap can be slow or even infinitely slow. Thus, after patches are formed, their two-dimensional suspension might stay practically stable. A factor that may enhance the formation of such a metastable state is a low boundary tension, \( \gamma \). On the other hand, at large values of \( \gamma \), patches will tend to unite in order to reduce the length of their boundary in contact with the solution. Therefore, the presence of a substance that helps to lower \( \gamma \) might help to stabilize the patches and thus the number of capped cells is lowered. It should be checked if substances that might reduce the percentage of cells undergoing capping (e.g., colchicine, cytochalasin B, or various metabolic inhibitors) do not affect the boundary tension between patches of various receptors and their solution. These materials might also cause changes in the membrane topography (which is not strictly a planar domain) and so influence the metastable equilibrium.

The cell or the membrane motion or their dynamic activity might help to abolish the possible metastable equilibrium of the "fog" formed by the patches. Substances like cytochalasin B and colchicine (e.g., by impairing cytoplasmic structure) or metabolic inhibitors (e.g., by stopping the cell metabolism) might interfere with this activity and thus the destruction of this metastable equilibrium may be prevented.

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